Dynamic Strength of Lipid Membranes Exposed to Antimicrobial Peptides

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Cationic antimicrobial peptides provide the first line of defense in the innate immune response to bacterial infection. Due to their strong potency against a broad spectrum of microbes, there is tremendous potential for their development as novel antibiotics [1]. However, a lack of understanding of their mechanisms of action and toxicity severely limits the design of effective and safe medications. For example, it is unclear whether certain peptides disrupt the physical integrity of the plasma membrane of bacteria or penetrate across it to act on intracellular targets. Using advanced micropipette aspiration techniques, our approach is to probe the material properties of lipid bilayer vesicles and evaluate the molecular-to-mesoscale determinates of dynamic membrane strength under physical and chemical stress.

The failure of biological membranes is a stochastic process involving the nucleation and growth of a single aqueous pore in the fluid lipid bilayer. By stressing lipid vesicles with steady ramps of tension over a wide range of time scales (loading rates between 0.1 and 100 mN/m/s), the kinetic limitations and energy barriers to pore formation are revealed [2]. Under slow tension ramps, membrane failure follows the classical theory of cavitation of an unstable hole in a fluid film, limited only by the energy required to form the pore edge. However, an anomalous increase in bilayer strength under rapid stress application reveals that pore formation is preceded by the nucleation of a rare lipidic defect. When exposed to dilute solutions of the amphipathic antimicrobial peptide melittin, bilayers composed of phosphatidylcholine (PC) lipids are dramatically weakened. These effects are quantified with extreme sensitivity (1:10000 peptide:lipids) by a reduction in pore edge energy, scaling down linearly with peptide concentration. Commensurate with the hole-edge activity, we also find that the rate of defect nucleation and damping of hole fluctuations increase with melittin concentration, suggesting that melittin interacts with defects prior to opening a membrane hole.

Alternatively, cholesterol provides a protective resistance to host (eukaryotic) membranes against the potentially toxic effects of these potent antibiotics. We find that cholesterol increases the edge energy of membrane pores and thereby reduces the destabilization induced by melittin. Cholesterols strengthening effects are strongly correlated to a reduction in bilayer permeability to water and an increase in elastic stretch modulus. The elastic strengthening is understood by an equilibrium model of tension-dependent condensing interactions between cholesterol and the lipid acyl chains. Melittin, on the other hand, has no effect on bilayer elasticity suggesting that its mechanisms of action include interactions at the bilayer-water interface and the pore edge (lipid headgroups), but not in the hydrophobic core (lipid chains).

At this point it is unclear whether these conclusions reflect a universal behavior of all cationic antimicrobial peptides, or are specific to α-helical peptides like melittin. There are four structural classes of cationic antimicrobials: (1) α-helical peptides, (2) β-sheet peptides stabilized by two to four disulfide bridges, (3) extended peptides rich in glycine, proline, tryptophan and/or histadine, and (4) loop peptides cyclized by a single disulphide bridge. We are currently investigating peptides representative of each of these classes to identify the relations between structure and the mechanisms of action through their effects on dynamic bilayer strength, elasticity and permeability.

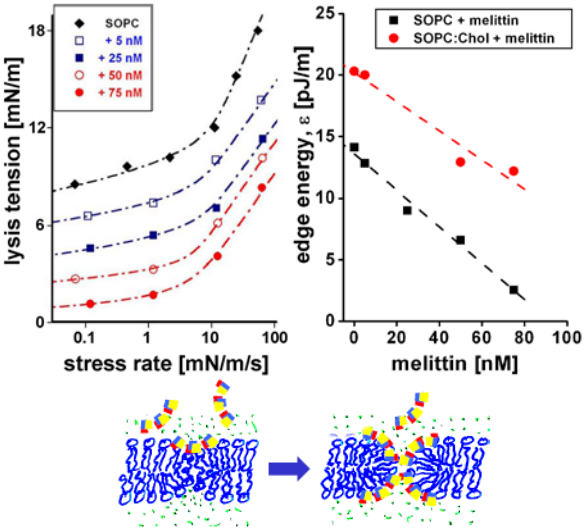

Fig. 1. Dynamic tension spectroscopy of SOPC lipid vesicles exposed to dilute concentrations of the antimicrobial peptide melittin. The most probable lysis tensions (*left*) are reduced at all stress loading rates by peptide concentrations as low as 5 nM. Pore edge energy (*right*), the strongest determinant of bilayer dynamic strength, decreases linearly with peptide concentration, extrapolating to zero (i.e. no mechanical strength) at 90-100 nM. Cholesterol provides resistance to pore formation and membrane failure by tension and antimicrobial peptides.

References

[1] Hancock, R.E.W. and H.-G. Sahl, 2006. *Nat. Biotechnol*. 24(12):1551-1557.
[2] Evans, E. *et al*. 2003. *Biophys. J*. 85(4):2342-2350.